Robust molecular micro-capsules for encapsulating and releasing hydrophilic contents.

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The hydrophobic-amphiphilic self-assembly approach has been employed to prepare molecular micro-capsules simply by cooling down an emulsion of a melted compound having two very well-distinguished units, both with highly non-polar and hydrophobic characteristics. The resulting micro-capsules are very stable and robust both in suspension and in dry conditions. Further, such micro-capsules can effectively encapsulate hydrophilic compounds which can later on be easily released upon the application of UV-light.

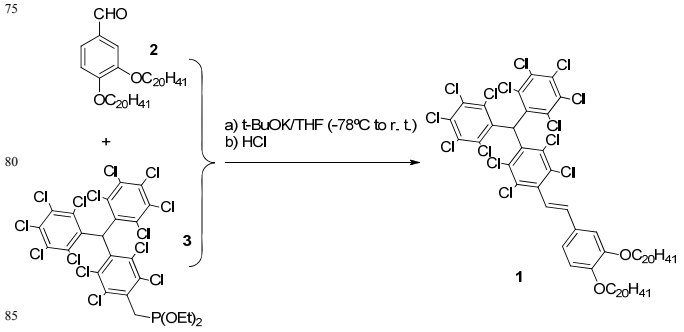
A key focus of attention in nanoscience is currently based on constructing large-scale systems of nano-structured materials that give rise to assemblies of high technological interest.[1] In such materials, the properties are mainly driven by the hierarchically organized assemblies, rather than the individual components. One particular case of micro-assemblies which raise an increasing significance are hollow spheres, or also called capsules. They allow for the encapsulation of materials to protect them from the environmental influences or for the confinement of chemical reactions. An unlimited number of examples of encapsulation can be found in nature, and the appeal of micro-capsules is expanded to a wide range of applications in the pharmaceutical, cosmetic, food, textile, adhesive and agricultural industries. Especially interesting are stimuli-responsive capsules (*i.e.*, smart capsules) that are able to release the encapsulated substances triggered by an external stimuli such as a pH change, light or temperature.[2]

It is well-known that organic-based capsules can be prepared by self-assembly of (phospho)lipids that aggregate in aqueous solutions owing to their amphiphilic nature forming bi-layered structures, so-called vesicles. These capsules can be further stabilized with polymerizable groups that give rise to interconnections between the molecules via covalent bonds and thus stabilize the shell-forming membrane.[3] However, in the most recent years, the use of macromolecules to prepare robust capsules have been more extensively explored employing a variety of techniques such as amphiphilicity, polyelectrolyte layer-by-layer, phase separation or polymerization.[4-5]

Very recently, the so-called "hydrophobic-amphiphilic" approach was developed to prepare molecular micro-scale objects with unprecedented shapes (*i.e.*, flowers, cones, fibers, etc).[6] This method was based on precipitating in a mixture of solvents a molecule bearing two very well-distinguished units, but both highly non-polar and hydrophobic, consisting of a fullerene derivatized with long alkyl chains. The van der Waals forces between the aliphatic chains and π···π interactions between the fullerene units determined the final assemblies. Later on, the method was applied to a different system: a perchlorotriphenylmethyl radical (PTM) moiety functionalised with three long alkyl chains.[7] In this case, the intermolecular Cl···Cl interactions of the PTM heads together with the CH_2···CH_2 interactions of the alkyl chains drove the formation of compact functional assemblies with unusual shapes.[8]

In this paper, we synthesize a new polychlorotriphenylmethane (αH-PTM) derivative, compound **1**, bearing two long alkyl chains at the *para* and *meta* positions of the phenylvinylene ring. This results in the lowering of the melting point of the compound down to 48 ºC, which is abnormal for a polychlorinated aromatic derivative, and the possibility of interdigitation of the alkyl chains in its supramolecular assemblies. We show that this material precipitates in a polar solution from an emulsion of its melted state leading to micro-capsules without the need of carrying out any additional step. Further, it is demonstrated that such assemblies are very stable and robust and can be exploited to encapsulate polar molecules, which can then be neatly released upon UV irradiation.

Compound **1** was synthesized in a 61 % yield by a Wittig-Horner-Emmons reaction between the corresponding benzaldehyde **2** and the αH-PTM phosphonate **3**[9] (Scheme 1).



Scheme 1. Synthesis of **1.**

The optimised procedure followed to prepare micro-capsules of **1** consisted of preparing a suspension (1.4 mM) in THF/water (2.5/1) and heating it at 65 ºC. This resulted in the melting of **1** and thus the formation of an emulsion in such a polar solvent media. After 5 minutes, the mixture was rapidly cooled down to room temperature leading to the final assemblies. This procedure is schematically depicted in Figure 1a.

To visualize the resulting supramolecular organizations, field emission scanning electron microscopy (FE-SEM) as well as transmission electron microscopy (TEM) images were acquired. Figure 2a shows the SEM images of the assemblies of **1** after being deposited on a silicon substrate, where spherical and

regular distributed hollow capsules can be clearly observed. Additionally, the TEM images of the particles deposited on a lacey carbon grid (Figure 2b) further confirmed their hollow nature. The capsules cortex have an approximate thickness of 60 nm, which corresponds to around 15 molecular layers considering the X-ray characterization (see below). The size and distribution of the capsules was estimated by light scattering (LS) giving an average diameter of 0.7 μm (Figure S1). It is important to highlight here that, opposite to conventional vesicles that are stable in the solution where they are produced but collapse immediately on solid supports after solvent evaporation, the capsules of **1** once formed are stable and robust even when the mother solution is dried.

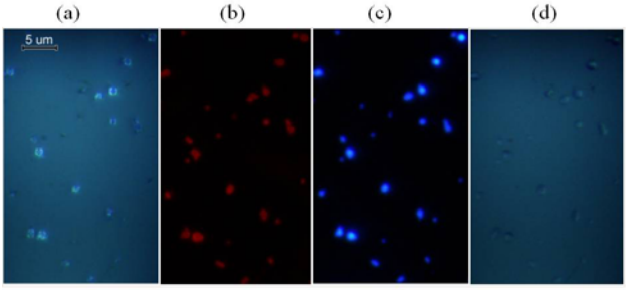


Figure 1. Schematic procedure followed to form the capsules (a) and to encapsulate the dye in them (b).

In order to gain a better understanding of the supramolecular structure of the micro-scale objects formed by **1** small-angle X-ray scattering (SAXS) characterization was performed. The SAXS spectra of the assemblies of **1** at room temperature give diffraction patterns that are attributed to the reflections (001) to (00n) planes with a *d* spacing value ca. 5.1 nm that indicates a lamellar layered structure (Figure S2). Taking into account the approximate length measured for the extended molecules (~42 Å) and the diameter of αH-PTM moieties (~10 Å), for every layer it is expected a complete interdigitation of the alkoxy chains and a face-to-face interaction between the αH-PTM moieties (Figure S3). Therefore, this confirms that the Cl···Cl and van der Waals interactions between the aliphatic chains are the driving force for the supramolecular ordering of the micro-capsules formed in such a polar solvent media.

To demonstrate the capability of the capsules of harboring hydrophilic guests, the red dies Rhodamine B or AlexaFluor-568® were dissolved in the THF/water mixture (2.5/1) (c=0.25 mM) in the micro-capsule formation process (Figure 1b). The

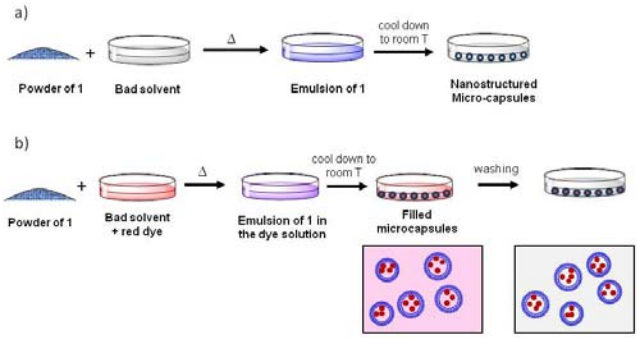


Figure 2. Top. SEM image of the capsules of **1** deposited on a silicon substrate. Inset. Zoom of the image showing with white arrows hollow capsules due to e-beam damage. Bottom. TEM image of the micro-capsules of **1** on a lacey carbon grid.

fabrication process was carried out as before, although an additional washing step was introduced at the end in order to remove the none-encapsulated dye. By optical fluorescence microscopy it is possible to observe the capsules and the dye taking advantage of the intrinsic fluorescence of **1** that shows a strong emission band at 465 nm when irradiated at 305 nm (Figure S4), and that of the red dye that emits at 550 nm (case of Rhodamine B, λ_{exc}= 510 nm) or 603 nm (AlexaFluor-568®, λ_{exc}= 510 nm). Figure 3 shows the fluorescence microscope images of the capsules filled with Rodhamine B once deposited on a quartz slide. The micro-capsules are visible with the transmitted white light. Once irradiated with green light (546 nm), the red fluorescence of the dye can be visualized in the same position where the capsules were seen. Further, the micro-capsules switched to blue when the UV-light (305 nm) was employed due to the emission of **1**. These results unambiguously confirmed the presence of the dye which was located in the same place as the micro-capsules. However, after exposure for 10 seconds to UV light, the capsules are shattered due to a photochemical cyclization leading to a phenanthrene derivative,[10] as indicated by MALDI-ToF analysis and UV-Vis measurements (Fig. S5).[11] As seen in the image taken after UV-irradiation (Figure 3d) the capsules are not structured anymore although some material is observed at the same positions where the original capsules were located. Thus, this external stimuli was exploited to release the content of the micro-capsules. The absorption and fluorescence spectra of the solvent from a suspension of micro-capsules of **1** filled with AlexaFluor-568® was registered before and after 10 seconds of UV irradiation at 305 nm. It was found that only after light irradiation the optical characteristics of the dye were detectable, confirming that it had been liberated (Figures S6-S7).

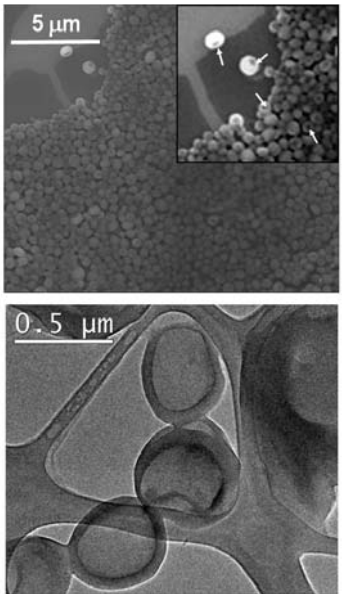


Figure 3. Microscope images of micro-capsules of **1** with: a) transmitted white light, b) irradiating with green light (mercury lamp, 546 nm), c) irradiating with UV light (mercury lamp, 305

nm) during 2 seconds and d) transmitted white light upon 10 s of irradiation at 305 nm.

To further demonstrate that the dye was encapsulated in the lumen of the micro-capsules and was not deposited around them, confocal microscopy experiments were carried out. These experiments were performed with AlexaFluor-568® due to its enhanced photostability. Figure 4 shows the confocal microscope images acquired at different planes separated by 0.04 μm using two channels. In this figure, images coming from the red channel (λ_{exc}= 561 nm; λ_{em}= 575-670 nm) and the blue channel (λ_{exc}= 476 nm; λ_{em}= 500-550 nm) have been overlapped. Notice that irradiating the sample at 476 nm did not damage the assemblies since compound **1** does not absorb at this wavelenght. These images clearly demonstrate that capsules have been successfully filled with a solution of the polar dye.

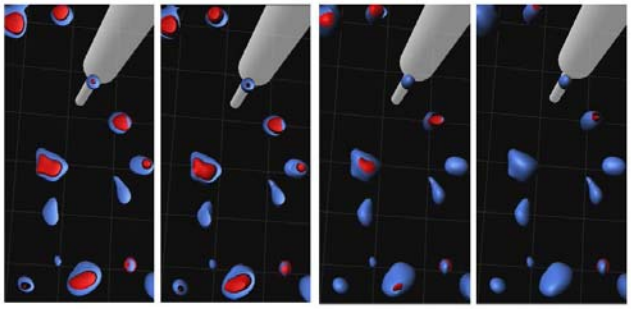


Figure 4. Superposition of image captures of some cross-sections of micro-capsules of **1** (blue) with AlexaFluor-568® in their lumen acquired with two channels: red channel (λ_{exc}= 561 nm; λ_{em}= 585-670 nm); Blue channel (λ_{exc}= 476 nm; λ_{em}= 500-550 nm).

In summary, it has been shown that the supramolecular hydrophobic-amphiphilic approach can be applied to fabricate robust molecular micro-capsules following a very simple process based on forming an emulsion of the melted compound **1** and solidifying it in a polar solvent media by lowering the temperature. Such micro-capsules can effectively encapsulate hydrophilic compounds which can later on be easily released upon the application of UV-light that shatters the micro-capsules. The fabrication of stable smart capsules based on molecules with the hydrophobic-amphiphilic approach offers an elegant alternative to the most common strategies currently used for preparing organic capsules which employs polymers or polymerizable monomers as building blocks, and thus brings novel perspectives into this field.

We thank the Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN); the DGI (Spain) with project POMAS CTQ2010–19501/BQU, and the Generalitat de Catalunya (grant 2009SGR00516). We also thank the European project ERC StG 2012-306826 e-GAMES and Mónica Roldán for the confocal microscopy experiments.

Notes and references

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† Electronic Supplementary Information (ESI) available: [General for the exprimental characterization of compounds and additional data.]. See DOI: 10.1039/b000000x/

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